Neuronal diversity: **Too many cell types for comfort?**

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Recent studies indicate that there are many more different types of neuron in the brain than previously thought. This richness will complicate life for those aiming to understand how the brain works – particularly for the neural modellers.

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Results that have recently come out of three laboratories will be seen as bad news by the more parsimonious among us: there are too many different types of neurons in the brain! The lowly retina, that highly specialized and very simple model for more interesting parts of the brain (Figure 1), has at least a dozen distinct types of ganglion cell [1] and more than two dozen types of amacrine cell [2]. And the hippocampus, the simplest of all cortices, has between two and four dozen types of inhibitory interneuron [3]. As we have no reason to believe that these simple neuronal circuits are unusually rich in cell types, such observations are very bad news for the micromodelers, those theorists who aim at explaining the brain's function in terms of detailed cellular interactions. At the same time, this great diversity represents a richness in brain circuits that is fitting for the world's most complex machine.

The new studies of the retina used clever high-tech approaches to obtain the large, unbiased samples of neurons needed for determining the number of cell types. DeVries and Baylor [1] exploited the multielectrode array, developed by Pine and adapted for retinal neurobiology by Meister [4], to record simultaneously from large numbers — sometimes approaching 100 neurons — of neighboring ganglion cells in the rabbit retina. At the same time, they stimulated the retina with computer-controlled light patterns that permitted the receptive fields and response properties of all the recorded cells to be determined simultaneously. Using these high-throughput methods, DeVries and Baylor [1] were able to assign cells to 11 distinct physiological classes. One distinguishing feature, for example, would be responsiveness to either the onset of light, defining an 'ON' cell, or to the onset of darkness, defining an 'OFF' cell.

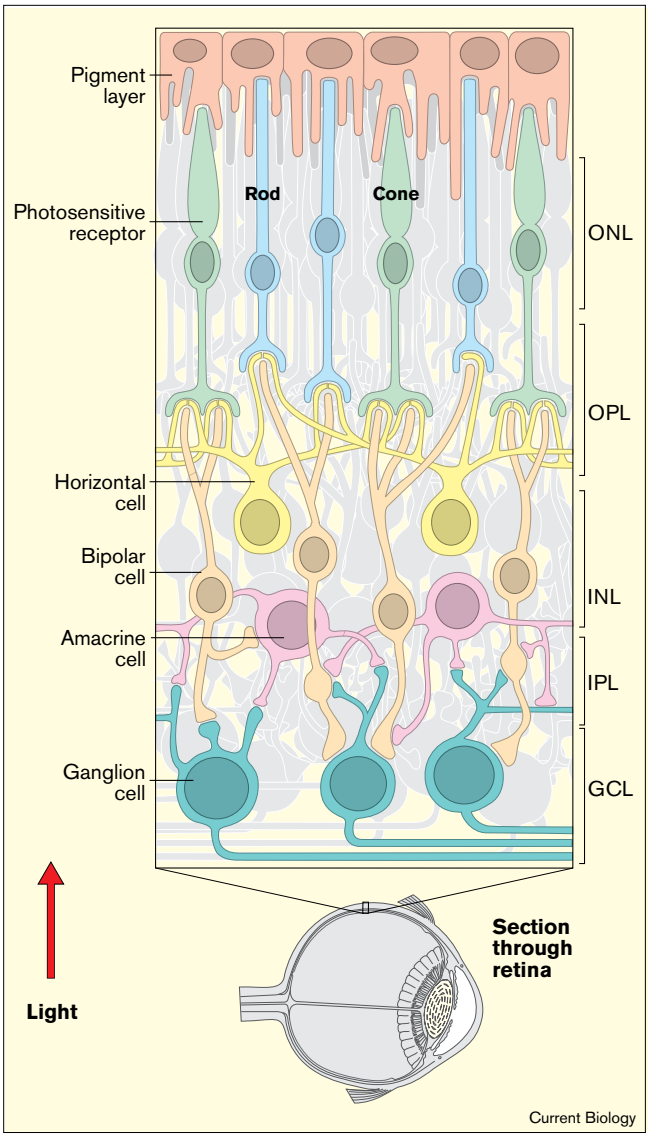
One of the most remarkable findings reported by DeVries and Baylor [1] is that each ganglion cell type individually tiles the retina. Starting with the initial observations of Wässle *et al.* [5], various workers had found that some particular ganglion cell types just cover the retina with their dendritic fields, but DeVries and Baylor are the first to demonstrate the generality of this tiling principle. 'Tiling' here means that the receptive fields belonging to any particular type of neighboring cell overlap only a little with other cells of the same type, and no parts of the retina fail to be covered by a receptive field of that cell type. This tiling is just what you would want: every spot in the retina, and thus in the visual field, is just covered by the dendrites of a ganglion cell type, but there is efficient use of the optic nerve in the sense that little overlapping information is transmitted to the brain.

MacNeil and Masland [2] studied the retina with a high-tech anatomical technique to obtain a large sample of amacrine cell dendritic morphologies. Older methods for studying amacrine cell morphology fell into three categories: the classical Golgi staining method; filling the cell with a dye, such as biocytin, through a microelectrode; and histochemical methods that rely on biochemical markers present in single neuronal types. The problem with all of these approaches is that they cannot give a large, unbiased sample of all amacrine cells present: the Golgi method is notoriously capricious; cells with small somata are hard to fill; and the histochemical methods depend on the existence of specific biochemical markers which are not known for most types of cell.

MacNeil and Masland [2] used the clever trick of staining all nuclei with a fluorescent dye (DAPI) and then illuminating the nucleus of a single amacrine cell to photoconvert a second dye with which the cells had been stained (dihydrorhodamine 123) from a non-fluorescent to a fluorescent form. Individual DAPI-stained nuclei could then be used to produce oxidation products of the second dye that filled the cell's dendritic tree so that it could be reconstructed with confocal microscopy. The reconstructed cells could then be classified according to their specific interactions with other (bipolar and ganglion) cells. Such interactions can be deduced from the positions of a cell's dendritic arbor, because the retina is such an orderly and highly organized laminar structure. For example, the terminals of ON and OFF types of bipolar cell are well segregated in different sublaminae. This method was validated by comparing the results with those for a few specific cell types amenable to the histochemical technique.

Using this approach to provide a large, fair sample of amacrine cells, MacNeil and Masland [2] found a minimum of 22 distinct morphological cell types. Cells

Figure 1



A cross section through the retina, showing the various cell types and layers referred to in the text. ONL, outer nuclear layer (photoreceptor cell bodies); OPL, outer plexiform layer (synapses between photoreceptors and bipolar or horizontal cells); INL, inner nuclear layer (bipolar and amacrine cell bodies); IPL, inner plexiform layer (synapses from bipolar and amacrine cells to ganglion cells); GCL, ganglion cell layer (ganglion cell bodies). Some amacrine cell bodies are actually in the INL, rather than the GCL – these are referred to as 'displaced amacrine cells'.

were classified according to the size of their dendritic tree, dendritic form (thin versus thick dendrites, for example), and dendritic stratification within the inner plexiform layer of the retina. A labeled population of over 250 amacrine cells, most of which could be classified (every class had to contain more than a single example and so cells in a class of their own were unassigned), was distributed more or less evenly between the two dozen classes. The single exception was the type designated A2, a previously well recognized amacrine type, which was over-represented at about 13% of the sample.

Most of the amacrine cell types described by MacNeil and Masland tile the retina, in the sense that the area covered by their dendritic tree times the number of cells of that type just about equals the entire retinal area. How close a cell comes to tiling in this sense is given by the 'coverage factor': a cell type whose coverage factor is 1 would just be able to cover the whole retina, whereas a cell type whose coverage factors is 100 could cover it 100 times over. The one cell type that does not just cover the retina is the starburst amacrine cell — a subtype of amacrine cell long recognized by its distinctive dendritic morphology — which has a coverage factor of over 100. This number seems outside the errors one might reasonable make in calculating coverage, suggesting that the starburst amacrines may need to be subdivided, perhaps based on physiological differences, into a lot more types.

The retina is embryologically a part of the brain and from the above studies it seems to have too many cell types; as many who work on cortex suspect, however, the retina may not be a typical part of the brain. Perhaps if we look at a more central brain region, such as the hippocampus, we can find some comfort for the lumpers over the splitters. No such comfort comes out of the recent work of Parra *et al.* [3], who have examined inhibitory cell types in the CA1 region of the hippocampus. These workers used standard, but very demanding, techniques to characterise a population of hippocampal inhibitory neurons. They found that these neurons can be divided into 16 morphological categories, a number not too different from the 22 amacrine cell types in the retina.

Parra *et al.* [3] examined the physiological characteristics of these inhibitory neurons, so they were also able to classify them in functional groups according to their spiking properties — whether they fired with a regular or burst pattern, for example — and the receptor types that they expressed — for example, whether they responded to serotonin. Of the 26 neurons for which all requisite information was available, no two fell into the same anatomical/functional category. Parra *et al.* have thus identified two dozen distinct types of inhibitory interneuron, and believe, based on partial information from a larger population of cells, that about four dozen types are actually present in the CA1 region of hippocampus.

All the information we have, then, supports the notion that there are a lot of types of neuron in the brain. How many might there be? If we use the principle that a particular type of neuron should tile the cortical surface, then we can find an upper limit for the number of cell types. Let's first consider inhibitory neurons in the hippocampus.

Underneath 1 mm^2 of hippocampal cortex in the CA1 region, one finds about 5×10^4 neurons, perhaps 10% or 20% of which are inhibitory. If we take the fraction of inhibitory neurons to be 0.1, then our standard piece of cortex will contain something like 5×10^3 inhibitory neurons. Suppose that each of these has a dendritic field that is 300 μm in diameter, so that, looking down on the cortical surface, its dendrites could cover an area of about 0.07 mm^2. It would take 14 neurons like this to cover our standard 1 mm^2 of cortex, and so there could be up to 350 types of inhibitory neurons because the available inhibitory neurons could tile the cortical surface 350 times over.

If we can accept the tiling principle clearly revealed in the retina, which is certainly a reasonable assumption, then there could be at least as many as 350 types of inhibitory neurons — or 700 types if 20% of all hippocampal neurons are inhibitory — a lot more than the 50 thought to be there by Parra *et al.* [3]. Thus the lower limit on the number of hippocampal inhibitory neuron types is the two to four dozen set by Parra *et al.* and the upper limit, from the tiling argument, is about an order of magnitude more than this. What is the real number of types? And why might the lower and upper limits be so far apart? The lower/upper limit gap may reveal the existence of multiple copies of neurons of each type; this redundancy could be used to make the brain a fault-tolerant computer. This argument is expanded a little below.

It is worth expanding this argument somewhat. Barlow [6] has pointed out that the optic nerve is a bottleneck through which all information about the visual world must be squeezed. This observation has been used to argue that the retina must have as little redundancy as possible, and this minimal redundancy argument can lead to an explanation for the form of ganglion cell receptive fields [7]. The cortical outputs are not limited to what can pass through a rather small optic nerve, however, and the constraints on cortical circuits may well not be the same as those in retina.

The cortex, then, might be able to afford to have multiple, nearly identical copies of a neuron. This would be advantageous because, if a few neurons die, the circuit could still work, and if individual neurons are variable in their behavior, the circuit could average over multiple copies of a single type to get more accurate information. Vertebrates have remarkably fault-tolerant brains that can still function at quite a high level even after relatively massive random loss of neurons (consider, for example, Presidents Eisenhower and Reagen). This fault tolerance must arise through redundancy of neuronal function. If we take the number of inhibitory hippocampal neuron types to be about 35, then the redundancy in inhibitory cells would be about 10, so that one could anticipate good continued function with a random loss of 10% or even 20% of the population.

This argument can be extended to the neocortex. Underneath 1 mm^2 of most regions of the primate cortical surface are about 10^5 neurons — the striate cortex is an exception with twice the number — each of which covers say 0.05 mm^2 with its dendritic arbor (assumed to be 0.25 mm in diameter). Twenty neurons with dendritic arbors of this size would be required to cover a square millimetre of cortex, so the upper limit on number of cell types, if each must tile the cortex, is $10^5/20$ = 5000, or an average of 1000 per layer. Now assume that the cortex has 10 times more neurons of each type than required to cover the cortex, a redundancy factor of 10 as guessed above for hippocampus: we thus would have about 100 neuron types per layer. If we believe there are a dozen ganglion cell types, two dozen amacrine cell types, and four dozen different kinds of inhibitory neurons in the CA1 region of hippocampus, 100 cell types per layer of neucortex seems like a reasonable number – not good news for the micromodelers.

These studies mark only the beginning of the task of determining how many cell types are needed to make our neural circuits work. If future studies continue to bear out these large numbers of cell types, the job of figuring out how the brain works will be a lot more complicated than the most optimistic of us thought.

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